Plasma riboflavin and vitamin B-6, but not homocysteine, folate or vitamin B-12 are inversely associated with breast cancer risk in the EPIC-Varese cohort[1,2,3,4]

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PubMed indexing: Agnoli, Grioni, Krogh, Pala, Allione, Matullo, Di Gaetano, Tagliabue, Pedraglio, Garrone, Cancarini, Cavalleri, Sieri

Word count: 5154; **Number of tables**: 3.

Running title: B vitamins and homocysteine in breast cancer risk.

[1] **Abbreviations.** BMI: body mass index; CI: confidence interval; CV: coefficient of variation; EPIC: European Prospective Investigation into Cancer and Nutrition; ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; ORDET: Hormones and Diet in the Etiology of Breast Cancer; PLP: pyridoxal-5'-phosphate; PR: progesterone receptor; RR: rate ratio.

[2] This study was supported by the Italian Ministry of Health.

[3] All authors declare no conflict of interest.

[4] Supplemental Table 1, 2, and 3 are available from the "Online Supporting Material" link in the online posting of the article.

ABSTRACT

Background. One-carbon metabolism – important for DNA stability and integrity – may play a role in breast carcinogenesis. However, epidemiological studies addressing this issue have yielded inconsistent results.

Objective. We prospectively investigated associations between breast cancer and plasma folate, riboflavin, vitamin B-6, vitamin B-12, and homocysteine, in women recruited to the Varese (Italy) cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC).

Methods. We performed a nested case-control study on women aged 35-65 years at recruitment, median body mass index 25.3 kg/m^2, who gave blood samples in 1987-1992, and again in 1993-1998. Breast cancer cases identified to 31 December 2009 were individually matched to controls. Relative risks (RRs) of breast cancer (and subtypes defined by hormone receptor status) with 95% confidence intervals (CIs) were estimated by unconditional logistic regression, controlling for matching factors and breast cancer risk factors.

Results. After a median of 14.9 years, 276 breast cancer cases were identified and matched to 276 controls. Increasing plasma vitamin B-6 was associated with decreased risk of overall (RR: 0.78; 95%CI: 0.63, 0.96 for 1SD increase), premenopausal (RR: 0.66; 95%CI: 0.48, 0.92 for 1SD increase), ER+ (RR: 0.79; 95%CI: 0.63, 1.00 for 1SD increase) and PR+ (RR: 0.72; 95%CI: 0.55, 0.95 for 1SD increase) breast cancers. Increasing plasma vitamin B-6 was also associated with decreased breast cancer risk in alcohol consumers (≥7 g/d) compared to consumption of <7 g/d plus non-consumption (RR: 0.71; 95% CI: 0.51, 0.99).

High plasma riboflavin was associated with significantly lower risk in premenopausal women (RR: 0.45; 95%CI: 0.21, 0.94 highest vs. lowest quartile, *P* trend=0.021). Plasma homocysteine, folate, and vitamin B-12 were not associated with breast cancer risk.

Conclusions. High plasma vitamin B-6 and riboflavin may lower breast cancer risk, especially in premenopausal women. Additional research is necessary to further explore these associations.

297 WORDS

Keywords: Breast cancer, B vitamins, homocysteine, nested case-control study, EPIC.

INTRODUCTION

The micronutrients folate, vitamin B-12, vitamin B-6, riboflavin, and homocysteine are all involved in one-carbon metabolism, and thus play important roles in maintaining DNA stability and integrity. Folate, as 5-methyltetrahydrofolate, is required to remethylate homocysteine to methionine which is converted to S-adenosylmethionine. The latter provides methyl groups for methylation reactions in general, and DNA and RNA biosynthesis in particular (1-5). S-adenosylmethionine depletion induces DNA hypomethylation which may lead to expression of proto-oncogenes and eventually cancer (6). Folate deficiency also results in deficient methylation of uracil to thymine, so that uracil is incorporated into DNA (2), leading to chromosome breaks and carcinogenesis (3,6). Vitamin B-12 deficiency is expected to cause chromosome breaks by the same mechanism as folate since it is an essential coenzyme in the methylation of homocysteine to methionine (2,4).

Vitamin B-6 is an essential coenzyme for several catabolic and anabolic reactions. In particular it is required for the conversion of tetrahydrofolate to 5,10-methylenetetrahydrofolate by serine hydroxymethyltransferase (2). 5,10-methylenetetrahydrofolate is required for the synthesis of nucleotides, themselves necessary for DNA synthesis and repair. Vitamin B-6 deficiency decreases the activity of serine hydroxymethyltransferase, thereby depleting the 5,10-methylenetetrahydrofolate pool, so that uracil is incorporated into DNA and chromosome breaks occur (2). Vitamin B-6 is also necessary for the synthesis of glutathione from homocysteine: glutathione is a cofactor of glutathione-S-transferases and peroxidases, which detoxify many carcinogenic compounds and protect against oxidative DNA damage (7-9). Riboflavin is the precursor of flavin adenine dinucleotide, a necessary cofactor for 5,10-methylenetetrahydrofolate reductase (10-13), which catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate: the latter being the methyl donor for DNA methylation (10,11,14,15).

Inadequate levels of folate, vitamin B-12, vitamin B-6, and riboflavin may all result in high levels of blood homocysteine (5,16) by disrupting the pathways summarized above (17). In vitro studies indicate that high homocysteine levels are associated with high proliferation rates of cancer cells including breast cancer cells (18,19), and also with oxidative damage to cells (20). High

homocysteine levels in blood have been associated with increased breast cancer risk in women with low folate status (21), and also in women with high body mass index (BMI), high plasma triglycerides, and abnormal oxidation of low-density lipoproteins (20,22-26) – all of which are associated with increased risk of certain cancers including breast cancer (27,28).

Studies on associations of plasma homocysteine (21,29-32), folate (29,30,32-34), vitamin B-12 (30,32,33), and vitamin B-6 (30,32,33) with breast cancer risk, have produced mixed results. To our knowledge no previous study has assessed the effect of plasma riboflavin on breast cancer risk. We carried out a case-control study, nested in the EPIC-Varese cohort, to prospectively evaluate whether plasma levels of homocysteine, folate, vitamin B-12, vitamin B-6, and riboflavin, were associated with risk of breast cancer, and risk of breast cancer subtypes defined by expression of hormone receptors.

MATERIALS AND METHODS

Study population and data collection

This was a case-control study nested in the women participating in the EPIC-Varese cohort study – part of the larger European Investigation into Cancer and Nutrition (EPIC). We considered 6071 women defined by the following eligibility criteria: recruitment to the prospective Hormones and Diet in the Etiology of Breast Cancer (ORDET) study in 1987-1992; recruitment to EPIC-Varese in 1993-1998 (70% of women who participated in ORDET were subsequently recruited in EPIC-Varese); and either premenopausal or postmenopausal at the ORDET and EPIC baselines (perimenopausal women and those with uncertain menopausal status were excluded).

The date of entry to the present study was the EPIC recruitment date. At EPIC baseline, after participants had given written informed consent, detailed information was collected on reproductive and medical history, physical activity, alcohol consumption, smoking, education and other socioeconomic variables using a standardized lifestyle questionnaire. Diet over the previous year was investigated using a food frequency questionnaire specifically developed to capture local dietary habits. Also at baseline, weight, height, and blood pressure were measured and a 30 mL

fasting blood sample was collected, using standardized procedures. The blood samples were divided into 0.5 mL aliquots of plasma, serum, red blood cells, and buffy coat, on the day of collection, and stored in liquid nitrogen at -196 °C (35).

All study participants had also been recruited to the earlier ORDET study and at ORDET baseline had given a blood sample. The stored plasma samples were analyzed and the results of these analyses were combined with those obtained from the samples collected at the EPIC baseline, so as to obtain mean estimates that were more reliable than those provided by a single measurement. The study protocol was approved by the ethics committee of the Fondazione IRCCS Istituto Nazionale dei Tumori (Milan, Italy).

Breast cancer cases and selection of control women

The 6071 women were followed-up to December 31, 2009 (median 14.9 years), through the Lombardy Cancer Registry, Varese Province, characterized by high data completeness and quality. A total of 276 new breast cancer cases were identified among the women over the follow-up period from the registry database. Information on estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (HER2) expression of the cancers was obtained from electronic pathology reports.

For each case, one matched control was chosen, using an incidence density sampling protocol, from appropriate risk sets consisting of cohort members alive and free of cancer at the time of diagnosis of the index case. Matching criteria were age at recruitment (±5 years), date of recruitment (±180 days), distance between ORDET and EPIC recruitment (±90 days), menopausal status (postmenopausal at both ORDET and EPIC baseline, premenopausal at ORDET baseline, postmenopausal at EPIC baseline, premenopausal at ORDET baseline and still premenopausal at EPIC baseline), and micronutrient analysis in the same batch.

Analysis of plasma samples

Analyses were performed both on EPIC and ORDET plasma samples. Folate and vitamin B-12 were determined on a Cobas 8000 modular analyzer (Roche Diagnostic GmbH) by Roche Elecsys electrochemoluminescence assays. Homocysteine was determined immuno-enzymatically as S-

adenosylhomocysteine produced from serum homocysteine on a Siemens Dimension Vista Lab System analyser (Siemens Healthcare Diagnostics Products GmbH). All assays were performed according to recommendations of the equipment manufacturers.

Levels of riboflavin and vitamin B-6 (the latter as pyridoxal-5'-phosphate − PLP − principal active form of vitamin B-6) were measured by LC/MS using a Thermo Fisher LCQ Vantage mass spectrometer coupled to a Thermo Fisher Scientific Transcend HPLC system. Perchloric acid was used to precipitate out proteins from aliquots of plasma to which had been added appropriate internal standards (^{15}N ^{13}C-labelled riboflavin; ^{2}H-labelled PLP). After incubation at 50°C for 10 minutes, the treated aliquots were filtered and injected into the HPLC system and eluted with a water, methanol, ammonium acetate gradient. The mass spectrometer was operated in single reaction monitoring mode to minimize interference from other compounds. Use of internal standards made it possible to correct for losses during purification and variation in instrument response. We excluded 17 cases and 18 controls because EPIC or ORDET plasma samples were not available.

Statistical analysis

We calculated plasma levels of homocysteine, folate, vitamin B-12, vitamin B-6, and riboflavin for each case and control as the mean of the values from the EPIC and ORDET samples. Coefficients of variation (CV) for each, considering the ORDET and EPIC samples as replicates of a single sample were as follows: 13% for homocysteine, 16% for folate, 14% for vitamin B-12, 31% for vitamin B-6, and 23% for riboflavin. Plasma levels were grouped into quartiles based on the distribution in controls. Baseline characteristics of study participants, according to quartiles of plasma vitamin B-6, were summarized as means and standard deviations (continuous variables) or frequencies (categorical variables). Unconditional logistic regression models were used to estimate relative risks (RRs) for breast cancer with 95% confidence intervals (CIs), with lowest quartile as reference; the significance of linear trends was assessed by treating each quartile as a continuous variable in the model and performing the Wald test. RRs were also calculated for 1 standard deviation increments of micronutrient concentration as a continuous variable. We ran a minimally

adjusted model, with the matching variables − age (continuous), date of recruitment (continuous), time between ORDET and EPIC recruitment (continuous), and menopausal status (premenopausal/postmenopausal in EPIC) − as covariates. Was also ran fully-adjusted models, with the following additional covariates: family history of breast cancer in first degree relatives (yes, no), age at menarche (<15 years, ≥15 years), parity (nulliparous, 1-2 children, >2 children), oral contraceptive use (never, sometime), education (≤8 years, >8 years), smoking status (never, former, current), alcohol consumption (continuous), and BMI (continuous).

We analyzed all women, and postmenopausal and premenopausal women separately. *P* values for interaction between plasma micronutrient with menopausal status were estimated by adding the product of quartile of plasma micronutrient with menopausal status to the model and applying the Wald test. We also analyzed risk of developing breast cancer subtypes defined by receptor status. Heterogeneity was investigated by the Wald test. We analyzed subgroups defined by alcohol intake (abstainers, <7 g/d, ≥7 g/d) with *P* interaction calculated by treating alcohol intake as a dichotomous variable (abstainer, consumer) and multiplying this by the analyte value (continuous). We excluded one case and two controls because confounder variables were missing; the analyses were thus performed on 514 women, 258 cases and 256 controls. All statistical tests were two-sided, differences were considered significant for $P < 0.05$. The analyses were performed with Stata version 11.2 (College Station, TX, USA).

RESULTS

Baseline characteristics of study participants by quartiles of plasma vitamin B-6 are shown in Table 1. Women in the highest vitamin B-6 quartile tended to have lower BMI, lower plasma homocysteine, and higher levels of other B vitamins. They were also better educated, and less likely to be smokers, to have used oral contraceptives, and to have a family history of breast cancer. Table 2 shows the risks (RRs) of developing breast cancer by quartiles of plasma homocysteine and B vitamins for all study women. High levels of vitamin B-6 in the continuous model were associated with decreased breast cancer risk [RR: 0.78; 95% CI: 0.63, 0.96 (fully-adjusted model)], however

no reduction in risk was found in the analysis based on quartiles. None of the other micronutrients was significantly associated with risk (*P* trend ≥0.31).

Table 3 shows risk estimates by menopausal status at baseline. Among postmenopausal women, none of the micronutrients was significantly associated with breast cancer risk (*P* trend ≥0.29). Among premenopausal women, high levels of vitamin B-6 were associated with significantly lowered breast cancer risk in the continuous model (RR: 0.66; 95% CI: 0.48, 0.92), however no reduction in risk was found in the analysis based on quartiles. The highest quartile of plasma riboflavin (compared to the lowest) was also associated with significantly lowered breast cancer risk [RR: 0.45; 95% CI: 0.21, 0.94, *P* trend 0.021 (fully adjusted model)]; there was a significant interaction between menopausal status and plasma riboflavin (*P*=0.021). Levels of homocysteine and the other B vitamins were not significantly associated with premenopausal breast cancer risk (*P* trend ≥0.22). However risk in the third quartile of vitamin B-12 concentration was significantly lower than reference (RR: 0.41; 95% CI: 0.19, 0.92). No interaction was found between menopausal status and plasma levels of homocysteine (*P*=0.44), folate (*P*=0.46), vitamin B-12 (*P*=0.45) or vitamin B-6 (*P*=0.42).

Associations of plasma homocysteine and B vitamins with breast cancer subtypes defined by hormonal receptor status are shown in Supplemental Tables 1, 2, and 3. The second quartile of plasma homocysteine was associated with significantly decreased risk of ER+ disease (RR: 0.54; 95% CI: 0.31, 0.96) compared to the lowest. Significant heterogeneity depending on ER status was found for plasma folate (*P* heterogeneity 0.045), however no significant association of folate with either ER+ (*P* trend=0.13) or ER- (*P* trend=0.24) disease was found. Significant heterogeneity depending on ER status was found for B-12 in the continuous model (*P* heterogeneity 0.032), again however no significant association was found with either ER+ or ER- disease. High vitamin B-6 was associated with lowered risk (borderline significance) of ER+ disease in the continuous model (RR: 0.79; 95% CI: 0.63, 1.00) (Supplemental Table 1). Vitamin B-6 in the continuous model was associated with a significantly lowered risk of PR+ disease (RR: 0.72; 95% CI: 0.55, 0.95). The second quartile of plasma homocysteine was associated with a significantly lowered (by 49%) risk

of PR+ disease, and the second quartile of vitamin B-12 was associated with a significantly decreased (by 61%) risk of PR- disease compared to the first quartile. No significant heterogeneity in relation to PR status was found (P ≥0.22) (Supplemental Table 2). None of the micronutrients analyzed was associated with the risk of developing either HER2+ (P trend ≥0.07) or HER2- (P trend ≥0.30) disease, and no significant heterogeneity in relation to HER2 status was found (P ≥0.09) (Supplemental Table 3).

When the analyses were stratified by alcohol intake, plasma vitamin B-12 was associated with significantly increased breast cancer risk among abstainers (RR: 4.88; 95% CI: 1.16, 20.55 for the highest vs. lowest quartile), however few cases were available for this sub-analysis; P for interaction between plasma vitamin B-12 and alcohol intake was not significant. High vitamin B-6 was associated with a significantly lowered breast cancer risk among women who drank >7g/d of alcohol (RR: 0.71; 95% CI: 0.51, 0.99 in the continuous model); no association was found for abstainers or women who drank ≤7g/d of alcohol. P for interaction between plasma vitamin B-6 and alcohol intake was not significant (0.87) (data not shown).

DISCUSSION

In this nested case-control study, considering all women, breast cancer risk decreased with increasing plasma vitamin B-6 levels. None of the other micronutrients was associated with breast cancer risk overall. However, when women were separated by menopausal status, high vitamin B-6 and riboflavin were associated with significantly decreased breast cancer risk among premenopausal women. Although plasma folate and vitamin B-12 were not significantly associated with the risk of breast cancer subtype defined by ER status, there was significant heterogeneity, with a non-significantly decreased risk of ER+ disease for high folate and vitamin B-12, and non-significantly increased risk of ER- disease for high folate and vitamin B-12. For increasing levels of vitamin B-6, the risks of ER+ and PR+ breast cancer decreased significantly, but heterogeneity between receptor positive and negative status was not significant. Finally, increasing vitamin B-6

was associated with decreasing breast cancer risk among women who drank >7g/day of alcohol compared to those who drank less or abstained.

Previous studies on associations of plasma levels of nutrients involved in one-carbon metabolism with breast cancer risk focused mainly on homocysteine and folate. Of five studies concerned with homocysteine, two found no association (30,32), two case-control studies found that higher homocysteine levels were associated with increased breast cancer risk (29,31), and a case-control study nested in the Women's Health Study found that homocysteine levels were not associated with overall breast cancer risk, but among women with low folate status the risk was increased if homocysteine was high (21). We found no evidence that homocysteine influenced breast cancer risk.

Our finding of a null association between plasma folate and breast cancer risk is in agreement with: a nested case-control study in the Washington County serum bank (30); a case-control study conducted in Taiwan (29); and a case-control study on postmenopausal women nested in the Malmö Diet and Cancer cohort (34). Other studies found a decreased breast cancer risk for increasing blood folate levels (36,37), especially in women whose alcohol intake was high (≥15 g/d) (32). Finally, a case-control study nested in the Women's Health Study found no association of folate with overall breast cancer risk, but that high folate was unexpectedly associated with increased risks of premenopausal, ER-positive, and PR-positive breast cancer (33).

To our knowledge, only three studies have investigated plasma vitamin B-12 and breast cancer risk. One found no association (33), as did our study; another found that for high B-12 risks of overall and premenopausal breast cancer decreased (32); and the other found that risks decreased especially among women postmenopausal at recruitment (30).

Our finding that high vitamin B-6 was associated with significantly lowered overall breast cancer risk is not completely in line with the results of the nested case-control study of Zhang et al. (32) which found that among postmenopausal women the association was of borderline significance, but was significant among women who drunk less that 15 g/d of alcohol. Lurie et al (38) also found that postmenopausal women with high vitamin B-6 had lowered breast cancer risk, which appeared

limited to women with ER+, PR+ and ER+PR+ cancers. Finally, two nested case-control studies found no association between plasma vitamin B-6 and overall breast cancer risk (30,33).

Our finding that breast cancer risk was significantly lowered among premenopausal women with high compared to low plasma riboflavin appears unique, as we are aware of no other study to investigate riboflavin/breast cancer associations. The fact that the association was confined to premenopausal women is unexpected – but not too unexpected, given the numerous differences in terms of risk factors, prognosis and molecular biology, between breast cancer in pre- and post-menopausal women; it suggests the need for further research.

Vitamin B-6 and riboflavin may lower breast cancer risk through mechanisms other than one-carbon metabolism, since they are essential cofactors in numerous reactions central to human metabolism (39,40). In addition, vitamin B-6 has been shown to decrease oxidative stress, cell proliferation and angiogenesis, and to enhance immune function (39,41); while low vitamin B-6 concentrations have been associated with high levels of inflammatory markers (42). Certain carcinogens are metabolized by flavin-dependent enzymes, and the resulting metabolites may have either increased or decreased carcinogenicity (43). Some studies (reviewed in (44)) suggest that the risk of certain cancers is increased when riboflavin is deficient.

Furthermore, both riboflavin and vitamin B-6 are cofactors in the pathway by which tryptophan is degraded to kynurenines, and many products of this pathway are neuroactive compounds with immunomodulatory effects (45). The same pathway is stimulated by inflammatory molecules and is often systematically up-regulated during an active immune response (45,46). Since inflammation has been linked to increased overall and premenopausal breast cancer risk (47,48), vitamin B-6 and riboflavin status might be linked to breast cancer risk by inflammation-related mechanisms, perhaps involving the kynurenine pathway. However few other data are available to suggest mechanisms by which low riboflavin status can increase the risk of breast cancer – an association we found in premenopausal women.

Study strengths include: prospective design which rendered reverse causation unlikely as an explanation for the associations found; the availability of detailed information on lifestyle, dietary,

and anthropometric variables, which made it to possible to control for potential confounders; and the availability of two plasma samples taken approximately five years apart, which made it possible to analyze plasma micronutrient levels twice, providing estimates that are likely to be more reliable than a single measurement. A possible study limitation is that samples were collected, stored at -196°C (EPIC samples) or -80°C (ORDET samples), and analyzed up to 25 years later. There may have been differential decay of the analytes over that period. However unless analyte decay varied with initial concentration (which seems unlikely) this will not bias analyte-risk associations. A stability study conducted on vitamin B-6, vitamin B-12, and folic acid in plasma with EDTA, and riboflavin in whole blood, found that no large decline had occurred after 4 years of storage at -20°C (49). Moreover, the small number of breast cancers diagnosed will have decreased the power to detect associations, especially in subgroup analyses. Another limitation is that we performed multiple statistical comparisons that were not corrected for, thereby increasing the risk of erroneously rejecting null hypotheses. Finally, little data is available on biological mechanisms that could explain associations between low vitamin B-6 and riboflavin status and increased breast cancer risk, especially among premenopausal women.

To conclude, the findings of this case-control study nested in the EPIC-Varese cohort suggest that high plasma concentrations of vitamin B-6 may decrease the risk of breast cancer and particularly of ER+ breast cancer, and may also lower the risk in moderate-to-heavy drinkers (>7g/d alcohol). High plasma levels of riboflavin may decrease the risk of breast cancer in premenopausal but not postmenopausal women. Homocysteine and other the B vitamins investigated do not seem to influence breast cancer risk. Further research is required to elucidate the mechanisms by which B vitamins can influence the etiology of breast cancer.

ACKNOWLEDGEMENTS

We thank A. Evangelista and D. Del Sette for technical support, and Don Ward for help with the English. The authors' contributions were as follows: VK and SS designed the study; SP, AA, and GM developed the methodology; CA, SG, and SS analyzed and interpreted the data; CA, SG, VP,

VK, and SS wrote the manuscript; IC, AC, and GG provided essential materials. CA had primary responsibility for final content. All authors have read and approved the final manuscript.

REFERENCES

1. Scott JM & Weir DG. Folic acid, homocysteine and one-carbon metabolism: a review of the essential biochemistry. J Cardiovasc Risk 1998;5:223-7.

2. Ames BN. DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutat Res 2001;475:7-20.

3. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB, Ames BN. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A 1997;94:3290-5.

4. Cooper AJ. Biochemistry of sulfur-containing amino acids. Annu Rev Biochem 1983;52:187-222.

5. McNulty H & Scott JM. Intake and status of folate and related B-vitamins: considerations and challenges in achieving optimal status. Br J Nutr 2008;99 Suppl 3:S48-S54.

6. Duthie SJ, Narayanan S, Brand GM, Pirie L, Grant G. Impact of folate deficiency on DNA stability. J Nutr 2002;132:2444S-9S.

7. Hayes JD & McLellan LI. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. Free Radic Res 1999;31:273-300.

8. Brigelius-Flohe R. Tissue-specific functions of individual glutathione peroxidases. Free Radic Biol Med 1999;27:951-65.

9. Hayes JD & Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 1995;30:445-600.

10. Chen J, Gammon MD, Chan W, Palomeque C, Wetmur JG, Kabat GC, Teitelbaum SL, Britton JA, Terry MB, Neugut AI, Santella RM. One-carbon metabolism, MTHFR polymorphisms, and risk of breast cancer. Cancer Res 2005;65:1606-14.

11. Otani T, Iwasaki M, Hanaoka T, Kobayashi M, Ishihara J, Natsukawa S, Shaura K, Koizumi Y, Kasuga Y, Yoshimura K, Yoshida T, Tsugane S. Folate, vitamin B6, vitamin B12, and vitamin B2 intake, genetic polymorphisms of related enzymes, and risk of colorectal cancer in a hospital-based case-control study in Japan. Nutr Cancer 2005;53:42-50.

12. Mason JB. Biomarkers of nutrient exposure and status in one-carbon (methyl) metabolism. J Nutr 2003;133 Suppl 3:941S-7S.

13. Shrubsole MJ, Gao YT, Cai Q, Shu XO, Dai Q, Hebert JR, Jin F, Zheng W. MTHFR polymorphisms, dietary folate intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. Cancer Epidemiol Biomarkers Prev 2004;13:190-6.

14. Choi SW & Mason JB. Folate and carcinogenesis: an integrated scheme. J Nutr 2000;130:129-32.

15. Lewis SJ, Harbord RM, Harris R, Smith GD. Meta-analyses of observational and genetic association studies of folate intakes or levels and breast cancer risk. J Natl Cancer Inst 2006;98:1607-22.

16. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 1993;270:2693-8.

17. Perla-Kajan J, Twardowski T, Jakubowski H. Mechanisms of homocysteine toxicity in humans. Amino Acids 2007;32:561-72.

18. Sun CF, Haven TR, Wu TL, Tsao KC, Wu JT. Serum total homocysteine increases with the rapid proliferation rate of tumor cells and decline upon cell death: a potential new tumor marker. Clin Chim Acta 2002;321:55-62.

19. Wu LL & Wu JT. Hyperhomocysteinemia is a risk factor for cancer and a new potential tumor marker. Clin Chim Acta 2002;322:21-8.

20. Hogg N. The effect of cyst(e)ine on the auto-oxidation of homocysteine. Free Radic Biol Med 1999;27:28-33.

21. Lin J, Lee IM, Song Y, Cook NR, Selhub J, Manson JE, Buring JE, Zhang SM. Plasma homocysteine and cysteine and risk of breast cancer in women. Cancer Res 2010;70:2397-405.

22. Ozkan Y, Ozkan E, Simsek B. Plasma total homocysteine and cysteine levels as cardiovascular risk factors in coronary heart disease. Int J Cardiol 2002;82:269-77.

23. Frauscher G, Karnaukhova E, Muehl A, Hoeger H, Lubec B. Oral administration of homocysteine leads to increased plasma triglycerides and homocysteic acid-additional mechanisms in homocysteine induced endothelial damage? Life Sci 1995;57:813-7.

24. Blom HJ, Engelen DP, Boers GH, Stadhouders AM, Sengers RC, de Abreu R, TePoele-Pothoff MT, Trijbels JM. Lipid peroxidation in homocysteinaemia. J Inherit Metab Dis 1992;15:419-22.

25. El Khairy L, Ueland PM, Nygard O, Refsum H, Vollset SE. Lifestyle and cardiovascular disease risk factors as determinants of total cysteine in plasma: the Hordaland Homocysteine Study. Am J Clin Nutr 1999;70:1016-24.

26. Gatt A, Makris A, Cladd H, Burcombe RJ, Smith JM, Cooper P, Thompson D, Makris M. Hyperhomocysteinemia in women with advanced breast cancer. Int J Lab Hematol 2007;29:421-5.

27. Vona-Davis L, Howard-McNatt M, Rose DP. Adiposity, type 2 diabetes and the metabolic syndrome in breast cancer. Obes Rev 2007;8:395-408.

28. Xue F & Michels KB. Diabetes, metabolic syndrome, and breast cancer: a review of the current evidence. Am J Clin Nutr 2007;86:s823-s835.

29. Chou YC, Lee MS, Wu MH, Shih HL, Yang T, Yu CP, Yu JC, Sun CA. Plasma homocysteine as a metabolic risk factor for breast cancer: findings from a case-control study in Taiwan. Breast Cancer Res Treat 2007;101:199-205.

30. Wu K, Helzlsouer KJ, Comstock GW, Hoffman SC, Nadeau MR, Selhub J. A prospective study on folate, B12, and pyridoxal 5'-phosphate (B6) and breast cancer. Cancer Epidemiol Biomarkers Prev 1999;8:209-17.

31. Wu X, Zou T, Cao N, Ni J, Xu W, Zhou T, Wang X. Plasma homocysteine levels and genetic polymorphisms in folate metablism are associated with breast cancer risk in chinese women. Hered Cancer Clin Pract 2014;12:2.

32. Zhang SM, Willett WC, Selhub J, Hunter DJ, Giovannucci EL, Holmes MD, Colditz GA, Hankinson SE. Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. J Natl Cancer Inst 2003;95:373-80.

33. Lin J, Lee IM, Cook NR, Selhub J, Manson JE, Buring JE, Zhang SM. Plasma folate, vitamin B-6, vitamin B-12, and risk of breast cancer in women. Am J Clin Nutr 2008;87:734-43.

34. Ericson UC, Ivarsson MI, Sonestedt E, Gullberg B, Carlson J, Olsson H, Wirfalt E. Increased breast cancer risk at high plasma folate concentrations among women with the MTHFR 677T allele. Am J Clin Nutr 2009;90:1380-9.

35. Palli D, Berrino F, Vineis P, Tumino R, Panico S, Masala G, Saieva C, Salvini S, Ceroti M, Pala V, Sieri S, Frasca G, Giurdanella MC, Sacerdote C, Fiorini L, Celentano E, Galasso R, Decarli A, Krogh V. A molecular epidemiology project on diet and cancer: the EPIC-Italy Prospective Study. Design and baseline characteristics of participants. Tumori 2003;89:586-93.

36. Beilby J, Ingram D, Hahnel R, Rossi E. Reduced breast cancer risk with increasing serum folate in a case-control study of the C677T genotype of the methylenetetrahydrofolate reductase gene. Eur J Cancer 2004;40:1250-4.

37. Rossi E, Hung J, Beilby JP, Knuiman MW, Divitini ML, Bartholomew H. Folate levels and cancer morbidity and mortality: prospective cohort study from Busselton, Western Australia. Ann Epidemiol 2006;16:206-12.

38. Lurie G, Wilkens LR, Shvetsov YB, Ollberding NJ, Franke AA, Henderson BE, Kolonel LN, Goodman MT. Prediagnostic plasma pyridoxal 5'-phosphate (vitamin b6) levels and invasive breast carcinoma risk: the multiethnic cohort. Cancer Epidemiol Biomarkers Prev 2012;21:1942-8.

39. Komatsu S, Yanaka N, Matsubara K, Kato N. Antitumor effect of vitamin B6 and its mechanisms. Biochim Biophys Acta 2003;1647:127-30.

40. Rivlin RS. Riboflavin metabolism. N Engl J Med 1970;283:463-72.

41. Matsubara K, Komatsu S, Oka T, Kato N. Vitamin B6-mediated suppression of colon tumorigenesis, cell proliferation, and angiogenesis (review). J Nutr Biochem 2003;14:246-50.

42. Gori AM, Sofi F, Marcucci R, Giusti B, Franco GG, Abbate R. Association between homocysteine, vitamin B(6) concentrations and inflammation. Clin Chem Lab Med 2007;45:1728-36.

43. Webster RP, Gawde MD, Bhattacharya RK. Modulation of carcinogen-induced DNA damage and repair enzyme activity by dietary riboflavin. Cancer Lett 1996;98:129-35.

44. Powers HJ. Riboflavin (vitamin B-2) and health. Am J Clin Nutr 2003;77:1352-60.

45. Theofylaktopoulou D, Ulvik A, Midttun O, Ueland PM, Vollset SE, Nygard O, Hustad S, Tell GS, Eussen SJ. Vitamins B2 and B6 as determinants of kynurenines and related markers of interferon-gamma-mediated immune activation in the community-based Hordaland Health Study. Br J Nutr 2014;112:1065-72.

46. Chen Y & Guillemin GJ. Kynurenine pathway metabolites in humans: disease and healthy States. Int J Tryptophan Res 2009;2:1-19.

47. Rose DP & Vona-Davis L. Biochemical and molecular mechanisms for the association between obesity, chronic inflammation, and breast cancer. Biofactors 2014;40:1-12.

48. Sun CA, Wu MH, Chu CH, Chou YC, Hsu GC, Yang T, Chou WY, Yu CP, Yu JC. Adipocytokine resistin and breast cancer risk. Breast Cancer Res Treat 2010;123:869-76.

49. Ocke MC, Schrijver J, Obermann-de Boer GL, Bloemberg BP, Haenen GR, Kromhout D. Stability of blood (pro)vitamins during four years of storage at -20 degrees C: consequences for epidemiologic research. J Clin Epidemiol 1995;48:1077-85.

Table 1. Baseline characteristics of study participants by quartiles of plasma vitamin B-6 among women of the EPIC-Varese study[1].

Characteristic	1: 1.998-6.723 ng/mL (n=136)	2: 6.724-9.438 ng/mL (n=132)	3: 9.439-13.13 ng/mL (n=127)	4: 13.14-109.3 ng/mL (n=119)
Age, years	53.1 ± 7.4	53.7 ± 7.7	53.5 ± 8.5	53.1 ± 8.6
Body mass index, kg/m^2	27.0 ± 4.7	25.6 ± 4.0	26.4 ± 5.0	25.5 ± 5.8
Alcohol consumption, g/d	7.4 ± 10.1	9.7 ± 11.7	9.6 ± 13.5	7.5 ± 11.5
Plasma homocysteine, mmol/L	11.5 ± 3.9	11.7 ± 4.5	11.8 ± 7.0	10.9 ± 3.0
Plasma folate, ng/mL	6.6 ± 1.7	7.1 ± 1.9	7.7 ± 2.1	8.0 ± 2.3
Plasma vitamin B-12, pg/mL	539.1 ± 177.7	602.8 ± 236.4	630.4 ± 337.2	605.2 ± 223.5
Plasma riboflavin, ng/mL	7.4 ± 5.8	8.1 ± 7.2	8.0 ± 6.7	9.6 ± 12.1
Family history of breast cancer, n (%)				
No	121 (89.0)	123 (93.2)	115 (90.5)	109 (91.6)
Yes	15 (11.0)	9 (6.8)	12 (9.5)	10 (8.4)
Age at menarche, n(%)				
<15 years	123 (90.4)	117 (88.6)	112 (88.2)	109 (91.6)
≥15 years	13 (9.6)	15 (11.4)	15 (11.8)	10 (8.4)
Menopausal status, n(%)				
Postmenopausal	69 (50.7)	75 (56.8)	72 (56.7)	62 (52.1)
Premenopausal	67 (49.3)	57 (43.2)	55 (43.3)	57 (47.9)
Parity, n(%)				
Nulliparous	6 (4.4)	17 (12.9)	17 (13.4)	11 (9.2)
1-2 children	92 (67.7)	87 (65.9)	77 (60.6)	84 (70.6)
>2 children	38 (27.9)	28 (21.2)	33 (26.0)	24 (20.2)
Oral contraceptive use, n(%)				
Never	88 (64.7)	92 (69.7)	89 (70.1)	70 (58.8)
Sometime	48 (35.3)	40 (30.3)	38 (29.9)	49 (41.2)
Education, n(%)				
≤ 8 years	66 (48.5)	62 (47.0)	55 (43.3)	43 (36.1)
>8 years	70 (51.5)	70 (53.0)	72 (56.7)	76 (63.9)
Smoking status, n(%)				
Current smoker	29 (21.32)	20 (15.2)	17 (13.4)	14 (11.8)
Ex-smoker	24 (17.7)	19 (14.4)	16 (12.6)	17 (14.3)
Never smoker	83 (61.0)	93 (70.4)	94 (74.0)	88 (73.9)

[1]Values are means ± SD, or n (%).

Table 2. RRs of developing breast cancer by quartiles of plasma homocysteine, folate, vitamin B-12, vitamin B-6, and riboflavin among women of the EPIC-Varese study.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* trend	Continuous (for each 1 SD increase)
Homocysteine						
Range, mmol/L	5.971-9.541	9.542-10.44	10.45-12.21	12.22-82.70		
Cases/Controls	74/64	47/64	74/65	63/63		
RR (95%CI) [1]	1	0.61 (0.37-1.02)	0.97 (0.59-1.59)	0.82 (0.49-1.38)	0.81	1.11 (0.90-1.36)
RR (95%CI) [2]	1	0.62 (0.37-1.05)	0.95 (0.58-1.56)	0.79 (0.46-1.34)	0.68	1.10 (0.89-1.36)
Folate						
Range, ng/mL	2.609-5.968	5.969-7.218	7.219-8.599	8.600-15.35		
Cases/Controls	79/64	64/64	59/64	56/64		
RR (95%CI) [1]	1	0.80 (0.50-1.30)	0.79 (0.48-1.28)	0.73 (0.44-1.19)	0.21	0.89 (0.75-1.06)
RR (95%CI) [2]	1	0.80 (0.49-1.31)	0.86 (0.52-1.44)	0.74 (0.45-1.23)	0.31	0.89 (0.75-1.07)
Vitamin B-12						
Range, pg/mL	175.4-443.2	443.3-545.4	545.5-687.8	687.9-2310		
Cases/Controls	76/64	55/64	60/64	67/64		
RR (95%CI) [1]	1	0.72 (0.44-1.18)	0.77 (0.47-1.26)	0.90 (0.55-1.46)	0.72	1.03 (0.86-1.22)
RR (95%CI) [2]	1	0.67 (0.40-1.11)	0.69 (0.42-1.15)	0.88 (0.53-1.45)	0.62	1.04 (0.86-1.25)
Vitamin B-6						
Range, ng/mL	1.998-6.723	6.724-9.438	9.439-13.13	13.14-109.3		
Cases/Controls	72/64	68/64	63/64	55/64		
RR (95%CI) [1]	1	0.93 (0.56-1.51)	0.86 (0.53-1.40)	0.77 (0.47-1.27)	0.29	0.80 (0.65-0.99)
RR (95%CI) [2]	1	0.93 (0.57-1.549	0.87 (0.52-1.43)	0.76 (0.45-1.27)	0.28	0.78 (0.63-0.96)
Riboflavin						
Range, ng/mL	1.465-4.512	4.513-6.405	6.406-9.052	9.053-122.7		
Cases/Controls	74/64	66/64	56/65	62/63		
RR (95%CI) [1]	1	0.92 (0.56-1.49)	0.74 (0.45-1.21)	0.87 (0.53-1.42)	0.41	1.08 (0.89-1.30)
RR (95%CI) [2]	1	0.88 (0.54-1.45)	0.73 (0.44-1.21)	0.83 (0.50-1.39)	0.36	1.08 (0.88-1.33)

[1] Adjusted for age, menopausal status, recruitment date, and distance between ORDET and EPIC recruitment.
[2] Further adjusted for family history of breast cancer, age at menarche, parity, oral contraceptive use, smoking status, education, alcohol consumption, and BMI.

Table 3. RRs of developing breast cancer by quartiles of plasma homocysteine, folate, vitamin B-12, vitamin B-6, and riboflavin according to menopausal status among women of the EPIC-Varese study.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* trend	Continuous (for each 1 SD increase)
Postmenopausal women						
Homocysteine						
Range, mmol/L	5.971-9.541	9.542-10.44	10.45-12.21	12.22-82.70		
Cases/Controls	27/21	24/30	44/42	43/47		
RR (95%CI) [1]	1	0.58 (0.26-1.29)	0.80 (0.38-1.65)	0.66 (0.31-1.39)	0.47	1.04 (0.77-1.39)
RR (95%CI) [2]	1	0.63 (0.27-1.45)	0.77 (0.37-1.64)	0.66 (0.30-1.44)	0.43	1.05 (0.77-1.41)
Folate						
Range, ng/mL	2.609-5.968	5.969-7.218	7.219-8.599	8.600-15.35		
Cases/Controls	37/33	32/33	35/35	34/39		
RR (95%CI) [1]	1	0.85 (0.43-1.69)	0.93 (0.48-1.82)	0.79 (0.41-1.55)	0.58	0.95 (0.74-1.21)
RR (95%CI) [2]	1	0.85 (0.41-1.75)	1.15 (0.56-2.37)	0.82 (0.40-1.66)	0.77	0.95 (0.73-1.23)
Vitamin B-12						
Range, pg/mL	175.4-443.2	443.3-545.4	545.5-687.8	687.9-2310		
Cases/Controls	42/44	29/34	34/27	33/35		
RR (95%CI) [1]	1	0.89 (0.46-1.71)	1.27 (0.65-2.48)	1.02 (0.54-1.93)	0.44	1.11 (0.86-1.43)
RR (95%CI) [2]	1	0.83 (0.42-1.65)	1.13 (0.56-2.27)	0.88 (0.45-1.72)	0.91	1.04 (0.79-1.36)
Vitamin B-6						
Range, ng/mL	1.998-6.723	6.724-9.438	9.439-13.13	13.14-109.3		
Cases/Controls	36/33	36/39	37/35	29/33		
RR (95%CI) [1]	1	0.82 (0.43-1.60)	0.94 (0.48-1.83)	0.83 (0.42-1.67)	0.72	0.89 (0.69-1.14)
RR (95%CI) [2]	1	0.82 (0.41-1.64)	0.94 (0.47-1.88)	0.91 (0.43-1.89)	0.90	0.87 (0.68-1.13)
Riboflavin						
Range, ng/mL	1.465-4.512	4.513-6.405	6.406-9.052	9.053-122.7		
Cases/Controls	34/37	36/40	33/38	35/25		
RR (95%CI) [1]	1	1.02 (0.53-1.97)	0.95 (0.49-1.84)	1.56 (0.78-3.14)	0.29	1.33 (0.98-1.79)
RR (95%CI) [2]	1	0.97 (0.49-1.93)	0.92 (0.45-1.85)	1.59 (0.76-3.33)	0.29	1.36 (0.98-1.87)
Premenopausal women						
Homocysteine						
Range, mmol/L	5.971-9.541	9.542-10.44	10.45-12.21	12.22-82.70		
Cases/Controls	47/43	23/34	30/23	20/16		

RR (95%CI) [1]	1	0.61 (0.31-1.20)	1.13 (0.56-2.26)	1.05 (0.48-2.32)	0.71	1.20 (0.84-1.71)
RR (95%CI) [2]	1	0.59 (0.29-1.19)	1.12 (0.55-2.28)	1.05 (0.46-2.39)	0.72	1.25 (0.85-1.83)
Folate						
Range, ng/mL	2.609-5.968	5.969-7.218	7.219-8.599	8.600-15.35		
Cases/Controls	42/31	32/31	24/29	22/25		
RR (95%CI) [1]	1	0.76 (0.38-1.51)	0.65 (0.31-1.33)	0.67 (0.32-1.40)	0.22	0.83 (0.64-1.08)
RR (95%CI) [2]	1	0.70 (0.34-1.42)	0.61 (0.28-1.30)	0.66 (0.30-1.46)	0.24	0.83 (0.63-1.09)
Vitamin B-12						
Range, pg/mL	175.4-443.2	443.3-545.4	545.5-687.8	687.9-2310		
Cases/Controls	34/20	26/30	26/37	34/29		
RR (95%CI) [1]	1	0.51 (0.24-1.11)	0.42 (0.20-0.89)	0.70 (0.33-1.49)	0.34	0.96 (0.75-1.22)
RR (95%CI) [2]	1	0.49 (0.22-1.10)	0.41 (0.19-0.92)	0.81 (0.36-1.82)	0.57	1.06 (0.81-1.39)
Vitamin B-6						
Range, ng/mL	1.998-6.723	6.724-9.438	9.439-13.13	13.14-109.3		
Cases/Controls	36/31	32/25	26/29	26/31		
RR (95%CI) [1]	1	1.10 (0.54-2.26)	0.77 (0.37-1.60)	0.69 (0.33-1.46)	0.24	0.70 (0.51-0.97)
RR (95%CI) [2]	1	1.07 (0.50-2.27)	0.80 (0.36-1.74)	0.65 (0.30-1.42)	0.22	0.66 (0.48-0.92)
Riboflavin						
Range, ng/mL	1.465-4.512	4.513-6.405	6.406-9.052	9.053-122.7		
Cases/Controls	40/27	30/24	23/27	27/38		
RR (95%CI) [1]	1	0.85 (0.41-1.77)	0.56 (0.26-1.18)	0.48 (0.24-0.97)	0.025	0.93 (0.74-1.17)
RR (95%CI) [2]	1	0.85 (0.40-1.83)	0.58 (0.27-1.26)	0.45 (0.21-0.94)	0.021	0.92 (0.73-1.17)

[1] Adjusted for age, recruitment date, and distance between ORDET and EPIC recruitment.
[2] Further adjusted for family history of breast cancer, age at menarche, parity, oral contraceptive use, smoking status, education, alcohol consumption, and BMI.

Supplemental Table 1. RRs of developing breast cancer subtypes defined by ER status according to quartiles of plasma homocysteine, folate, vitamin B-12, PLP vitamin B-6 and riboflavin, among women recruited to the EPIC-Varese study

		ER+			ER-	
	Cases/Controls	RR (95%CI)[1]	RR (95%CI)[2]	Cases/Controls	RR (95%CI)[1]	RR (95%CI)[2]
Homocysteine						
Quartile 1 (low)	59/64	1	1	8/64	1	1
Quartile 2	33/64	0.54 (0.31-0.94)	0.54 (0.31-0.96)	12/64	1.39 (0.53-3.69)	1.43 (0.53-3.84)
Quartile 3	56/65	0.90 (0.54-1.52)	0.90 (0.53-1.53)	10/65	1.19 (0.43-3.32)	1.12 (0.40-3.15)
Quartile 4 (high)	50/63	0.81 (0.46-1.40)	0.79 (0.45-1.40)	11/63	1.30 (0.46-3.67)	1.21 (0.42-3.44)
P trend		0.77	0.71		0.75	0.89
P heterogeneity	0.63[1] /0.73[2]					
Continuous		1.09 (0.85-1.41)	1.10 (0.85-1.43)		1.08 (0.69-1.69)	1.05 (0.67-1.66)
P heterogeneity	0.95[1] /0.85[2]					
Folate						
Quartile 1 (low)	65/64	1	1	6/64	1	1
Quartile 2	49/64	0.75 (0.45-1.25)	0.72 (0.43-1.22)	11/64	1.83 (0.63-5.30)	1.95 (0.66-5.73)
Quartile 3	43/64	0.69 (0.41-1.16)	0.73 (0.42-1.27)	14/64	2.60 (0.93-7.27)	2.92 (1.01-8.48)
Quartile 4 (high)	41/64	0.65 (0.38-1.10)	0.65 (0.37-1.12)	10/64	1.75 (0.59-5.16)	1.83 (0.61-5.53)
P trend		0.09	0.13		0.26	0.24
P heterogeneity	0.043[1] /0.045[2]					
Continuous		0.86 (0.71-1.04)	0.86 (0.70-1.05)		1.06 (0.77-1.47)	1.08 (0.77-1.51)
P heterogeneity	0.22[1] /0.19[2]					
Vitamin B-12						
Quartile 1 (low)	60/64	1	1	12/64	1	1
Quartile 2	44/64	0.73 (0.43-1.24)	0.68 (0.40-1.17)	6/64	0.51 (0.18-1.45)	0.48 (0.17-1.40)
Quartile 3	45/64	0.75 (0.44-1.27)	0.68 (0.39-1.17)	12/64	0.95 (0.39-2.31)	0.97 (0.39-2.43)
Quartile 4 (high)	49/64	0.84 (0.50-1.41)	0.82 (0.48-1.41)	11/64	0.97 (0.39-2.38)	1.00 (0.39-2.55)
P trend		0.52	0.45		0.81	0.74
P heterogeneity	0.55[1] /0.46[2]					
Continuous		0.92 (0.74-1.13)	0.91 (0.73-1.14)		1.26 (0.93-1.71)	1.35 (0.96-1.88)
P heterogeneity	0.05[1] /0.032[2]					
Vitamin B-6						
Quartile 1 (low)	54/64	1	1	11/64	1	1
Quartile 2	55/64	1.01 (0.60-1.69)	1.01 (0.59-1.73)	10/64	0.88 (0.35-2.24)	0.88 (0.34-2.29)

Quartile 3	46/64	0.84 (0.49-1.42)	0.84 (0.48-1.45)	11/64	0.94 (0.38-2.36)	0.96 (0.37-2.49)
Quartile 4 (high)	43/64	0.80 (0.47-1.36)	0.77 (0.44-1.34)	9/64	0.82 (0.31-2.14)	0.83 (0.30-2.28)
P trend		0.32	0.27		0.73	0.28
P heterogeneity	0.83 [1] /0.74 [2]					
Continuous		0.82 (0.66-1.02)	0.79 (0.63-1.00)		0.77 (0.50-1.19)	0.74 (0.48-1.14)
P heterogeneity	0.79 [1] /0.76 [2]					
Riboflavin						
Quartile 1 (low)	55/64	1	1	15/64	1	1
Quartile 2	49/64	0.91 (0.54-1.54)	0.86 (0.50-1.47)	9/64	0.63 (0.25-1.56)	0.66 (0.26-1.65)
Quartile 3	40/65	0.72 (0.42-1.24)	0.69 (0.40-1.20)	12/65	0.76 (0.32-1.77)	0.80 (0.33-1.89)
Quartile 4 (high)	54/63	1.02 (0.61-1.71)	0.93 (0.55-1.60)	5/63	0.34 (0.12-1.01)	0.38 (0.13-1.15)
P trend		0.86	0.65		0.09	0.15
P heterogeneity	0.11 [1] /0.24 [2]					
Continuous		1.10 (0.90-1.34)	1.09 (0.88-1.36)		1.09 (0.81-1.47)	1.13 (0.83-1.54)
P heterogeneity	0.92 [1] /0.80 [2]					

[1] Adjusted for age, menopausal status, recruitment date, and distance between ORDET and EPIC recruitment.
[2] Further adjusted for family history of breast cancer, age at menarche, parity, oral contraceptive use, smoking status, education, alcohol consumption, and BMI.

Supplemental Table 2. RRs of developing breast cancer subtypes defined by PR status according to quartiles of plasma homocysteine, folate, vitamin B-12, PLP vitamin B-6 and riboflavin, among women recruited to the EPIC-Varese study

		PR+			PR-	
	Cases/Controls	RR (95%CI)[1]	RR (95%CI)[2]	Cases/Controls	RR (95%CI)[1]	RR (95%CI)[2]
Homocysteine						
Quartile 1 (low)	49/64	1	1	19/64	1	1
Quartile 2	25/64	0.50 (0.28-0.91)	0.51 (0.28-0.94)	21/64	1.01 (0.49-2.08)	1.02 (0.49-2.13)
Quartile 3	45/65	0.90 (0.52-1.56)	0.90 (0.51-1.58)	19/65	0.87 (0.41-1.84)	0.83 (0.39-1.78)
Quartile 4 (high)	39/63	0.80 (0.44-1.43)	0.80 (0.44-1.47)	20/63	0.87 (0.40-1.90)	0.80 (0.36-1.76)
P trend		0.77	0.78		0.65	0.49
P heterogeneity	0.83[1] /0.66[2]					
Continuous		1.10 (0.85-1.44)	1.13 (0.86-1.49)		1.02 (0.71-1.46)	0.99 (0.68-1.42)
P heterogeneity	0.66[1] /0.47[2]					
Folate						
Quartile 1 (low)	52/64	1	1	19/64	1	1
Quartile 2	38/64	0.73 (0.42-1.25)	0.70 (0.40-1.22)	22/64	1.14 (0.56-2.33)	1.15 (0.55-2.38)
Quartile 3	37/64	0.75 (0.43-1.29)	0.80 (0.45-1.42)	19/64	1.07 (0.51-2.23)	1.14 (0.53-2.44)
Quartile 4 (high)	31/64	0.62 (0.35-1.09)	0.60 (0.33-1.08)	19/64	1.00 (0.48-2.10)	1.04 (0.49-2.20)
P trend		0.11	0.13		0.97	0.93
P heterogeneity	0.26[1] /0.23[2]					
Continuous		0.84 (0.68-1.03)	0.83 (0.67-1.03)		1.00 (0.78-1.29)	1.02 (0.79-1.33)
P heterogeneity	0.21[1] /0.14[2]					
Vitamin B-12						
Quartile 1 (low)	45/64	1	1	26/64	1	1
Quartile 2	40/64	0.89 (0.51-1.54)	0.83 (0.47-1.47)	11/64	0.43 (0.19-0.95)	0.39 (0.18-0.88)
Quartile 3	36/64	0.79 (0.45-1.40)	0.71 (0.40-1.27)	20/64	0.77 (0.39-1.55)	0.74 (0.36-1.52)
Quartile 4 (high)	37/64	0.83 (0.47-1.46)	0.80 (0.45-1.43)	22/64	0.91 (0.46-1.79)	0.91 (0.45-1.84)
P trend		0.47	0.37		0.97	0.97
P heterogeneity	0.57[1] /0.48[2]					
Continuous		0.93 (0.74-1.15)	0.92 (0.73-1.16)		1.09 (0.84-1.42)	1.15 (0.87-1.52)
P heterogeneity	0.26[1] /0.16[2]					
Vitamin B-6						
Quartile 1 (low)	47/64	1	1	18/64	1	1
Quartile 2	43/64	0.91 (0.53-1.56)	0.90 (0.51-1.58)	21/64	1.12 (0.54-2.33)	1.08 (0.51-2.28)

Quartile 3	34/64	0.72 (0.41-1.26)	0.70 (0.39-1.26)	22/64	1.18 (0.57-2.44)	1.18 (0.56-2.46)
Quartile 4 (high)	34/64	0.72 (0.41-1.26)	0.67 (0.37-1.21)	18/64	1.03 (0.49-2.18)	1.02 (0.47-2.24)
P trend		0.17	0.13		0.90	0.89
P heterogeneity	0.27 [1] /0.22 [2]					
Continuous		0.76 (0.58-0.99)	0.72 (0.55-0.95)		0.90 (0.70-1.15)	0.86 (0.66-1.12)
P heterogeneity	0.31 [1] /0.31 [2]					
Riboflavin						
Quartile 1 (low)	46/64	1	1	22/64	1	1
Quartile 2	35/64	0.78 (0.45-1.38)	0.72 (0.40-1.28)	23/64	1.07 (0.54-2.14)	1.12 (0.55-2.26)
Quartile 3	32/65	0.70 (0.39-1.24)	0.65 (0.36-1.18)	20/65	0.85 (0.42-1.73)	0.90 (0.43-1.86)
Quartile 4 (high)	45/63	1.01 (0.59-1.74)	0.91 (0.51-1.60)	14/63	0.66 (0.31-1.41)	0.70 (0.32-1.54)
P trend		0.94	0.70		0.25	0.35
P heterogeneity	0.31 [1] /0.55 [2]					
Continuous		1.19 (0.96-1.47)	1.18 (0.94-1.48)		0.83 (0.56-1.23)	0.88 (0.59-1.30)
P heterogeneity	0.08 [1] /0.14 [2]					

[1] Adjusted for age, menopausal status, recruitment date, and distance between ORDET and EPIC recruitment.
[2] Further adjusted for family history of breast cancer, age at menarche, parity, oral contraceptive use, smoking status, education, alcohol consumption, and BMI.

Supplemental Table 3. RRs of developing breast cancer by HER2 subtype by quartiles of plasma homocysteine, folate, vitamin B-12, vitamin B-6 and riboflavin among women recruited to the EPIC-Varese study.

		HER2+			HER2-	
	Cases/Controls	RR (95%CI)[1]	RR (95%CI)[2]	Cases/Controls	RR (95%CI)[1]	RR (95%CI)[2]
Homocysteine						
Quartile 1 (low)	15/64	1	1	46/64	1	1
Quartile 2	7/64	0.43 (0.16-1.13)	0.44 (0.16-1.19)	32/64	0.67 (0.38-1.20)	0.67 (0.37-1.21)
Quartile 3	7/65	0.46 (0.17-1.24)	0.40 (0.15-1.10)	46/65	0.96 (0.55-1.67)	0.94 (0.53-1.66)
Quartile 4 (high)	7/63	0.46 (0.16-1.28)	0.40 (0.14-1.17)	42/63	0.88 (0.49-1.58)	0.86 (0.47-1.57)
P trend		0.12	0.07		0.92	0.85
P heterogeneity	0.15 [1] /0.09 [2]					
Continuous		1.06 (0.66-1.71)	1.08 (0.65-1.79)		1.00 (0.75-1.33)	1.00 (0.74-1.34)
P heterogeneity	0.80 [1] /0.77 [2]					
Folate						
Quartile 1 (low)	10/64	1	1	47/64	1	1
Quartile 2	5/64	0.51 (0.16-1.59)	0.51 (0.16-1.64)	46/64	0.96 (0.56-1.65)	0.91 (0.53-1.59)
Quartile 3	10/64	1.11 (0.43-2.89)	1.30 (0.48-3.58)	38/64	0.86 (0.49-1.50)	0.91 (0.51-1.62)
Quartile 4 (high)	11/64	1.17 (0.46-2.98)	1.26 (0.47-3.35)	35/64	0.77 (0.44-1.36)	0.77 (0.43-1.38)
P trend		0.49	0.38		0.33	0.41
P heterogeneity	0.23 [1] /0.19 [2]					
Continuous		1.11 (0.80-1.55)	1.13 (0.80-1.58)		0.88 (0.72-1.08)	0.88 (0.71-1.08)
P heterogeneity	0.18 [1] /0.17 [2]					
Vitamin B-12						
Quartile 1 (low)	11/64	1	1	50/64	1	1
Quartile 2	7/64	0.67 (0.24-1.84)	0.61 (0.21-1.74)	36/64	0.72 (0.41-1.26)	0.65 (0.37-1.15)
Quartile 3	8/64	0.69 (0.25-1.84)	0.54 (0.19-1.53)	38/64	0.76 (0.43-1.32)	0.66 (0.37-1.17)
Quartile 4 (high)	10/64	0.92 (0.36-2.35)	0.89 (0.33-2.39)	42/64	0.86 (0.50-1.48)	0.81 (0.46-1.42)
P trend		0.87	0.74		0.63	0.45
P heterogeneity	0.92 [1] /0.94 [2]					
Continuous		1.08 (0.76-1.54)	1.11 (0.75-1.65)		0.98 (0.79-1.21)	0.98 (0.78-1.22)
P heterogeneity	0.59 [1] /0.53 [2]					
Vitamin B-6						
Quartile 1 (low)	12/64	1	1	45/64	1	1
Quartile 2	9/64	0.72 (0.28-1.83)	0.71 (0.27-1.88)	45/64	1.00 (0.58-1.72)	1.02 (0.58-1.79)

Quartile 3	9/64	0.70 (0.27-1.80)	0.67 (0.25-1.81)	39/64	0.86 (0.49-1.50)	0.88 (0.49-1.56)
Quartile 4 (high)	6/64	0.49 (0.17-1.42)	0.43 (0.14-1.30)	37/64	0.84 (0.48-1.47)	0.84 (0.47-1.50)
P trend		0.20	0.14		0.45	0.47
P heterogeneity	0.40 [1] /0.29 [2]					
Continuous		0.77 (0.48-1.23)	0.68 (0.41-1.11)		0.81 (0.64-1.03)	0.81 (0.63-1.02)
P heterogeneity	0.81 [1] /0.51 [2]					
Riboflavin						
Quartile 1 (low)	10/64	1	1	48/64	1	1
Quartile 2	9/64	0.94 (0.35-2.49)	0.88 (0.32-2.40)	44/64	0.95 (0.55-1.63)	0.90 (0.51-1.57)
Quartile 3	10/65	0.97 (0.38-2.53)	0.98 (0.36-2.63)	32/65	0.66 (0.37-1.17)	0.62 (0.34-1.12)
Quartile 4 (high)	7/63	0.72 (0.26-2.02)	0.76 (0.26-2.23)	42/63	0.91 (0.52-1.56)	0.82 (0.46-1.44)
P trend		0.59	0.71		0.47	0.30
P heterogeneity	0.90 [1] /0.85 [2]					
Continuous		0.95 (0.61-1.49)	0.99 (0.67-1.48)		1.14 (0.93-1.40)	1.13 (0.91-1.41)
P heterogeneity	0.42 [1] /0.50 [2]					

[1] Adjusted for age, menopausal status, recruitment date, and distance between ORDET and EPIC recruitment.
[2] Further adjusted for family history of breast cancer, age at menarche, parity, oral contraceptive use, smoking status, education, alcohol consumption, and BMI.